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Minireview

Current challenges in quantitative modeling of epidermal growth factor signaling

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Abstract Over the last decade, epidermal growth factor (EGF) signaling has been used repeatedly as a testbed for pioneering computational systems biology. Recent breakthroughs in our molecular understanding of EGF signaling pose new challenges for mathematical modeling strategies. Three key areas emerge as particularly relevant: the pervasive importance of compartmentalization and endosomal trafficking; the complexity of signalosome complexes; and the regulatory influence of diffusion and spatiality. Each one of them demands a drastic change in current computational approaches. We discuss recent developments in the field that address these emerging aspects in a new generation of more realistic – and potential more useful – models of EGF signaling.

Keywords: Epidermal growth factor; Computational modeling; Systems biology

1. Introduction

Epidermal growth factor (EGF) signaling is one of the best-studied cellular communication pathways. It is widely employed in animal organisms, where it stimulates cell proliferation, regulates differentiation, and determines cell fate decisions in morphogenesis. Because of its pro-mitotic potential, it is also an important contributor in many malignant diseases. At the same time, EGF signaling provides one of the central paradigms of systems biology. It is one of the few biological systems where computational modeling has contributed significantly to the elucidation of the molecular machinery (reviewed in [1,2]). It has attracted special attention because it is one of a few systems that are both sufficiently well-known and sufficiently complex to feature interesting emergent behavior. Emergent behavior is one of the key motivations of a systems biology approach. It is defined as complex behavior that can not be predicted from the properties of the system components in isolation, but only "emerges" when they are put together in a functional whole. By definition such properties can only be discovered by looking at a concrete instantiation of the integrated system, rather than by the reductionist analysis of its parts. Computational models facilitate this integrated, comprehensive analysis, and a well-defined model system such as the EGF pathway is particularly useful to examine the challenges and potentials of this approach.

2. The biochemical basis of EGF signaling

The proper response of cells to extracellular growth factor signals requires a complex orchestration of cellular events that sense, interpret, transmit, and terminate the signals (reviewed in [3–6]; and with a special focus on model organisms in [7] (*Drosophila*) and [8] (*Caenorhabiditis elegans*)). Transmembrane receptors like EGF receptor play a key role, not only in initiating, but also in integrating these processes. After binding to its extracellular ligand the EGF receptor undergoes conformational changes that allow it to homo-dimerize and subsequently *trans*-phosphorylate its binding partner at specific tyrosine residues in the cytoplasmic tails of the protein (auto-phosphorylation). The resulting phospho-tyrosines serve as specific docking sites for a variety of effector proteins containing SH2 or PTB domains, most notably GAP, Grb2, and Shc, leading to the assembly of distinct signaling complexes on the activated receptors.

In the classical pathway, the assembled complexes in consequence transmit the signal to a protein kinase cascade (Ras-Raf-MEK-Erk) that ultimately leads to the activation of the final effector proteins. Depending on cell type and physiological state, equally important aspects of the signal may be transmitted via, e.g., phospholipase gamma and protein kinase C, which also provide opportunities for cross-talk with other signaling pathways.

At the same time, however, while signaling complex proteins are still being recruited to positively convey the signal into the cell, negative signaling events are initiated that will attenuate the amplitude and duration of the signal. The most obvious of these is the rapid internalization of activated receptor into endosomes, which is triggered by specific receptor ubiquitination events that in general target the receptor for inactivation and destruction in the lysosome. This process is itself highly regulated and involves the assembly and activation of specific internalization and trafficking complexes. Interestingly, the activated receptors can be directed to different internalization routes and it has been suggested that signaling outcome and

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strength are dependent on the chosen entry route [9]. However, the molecular mechanisms that decide which entry pathway is taken by the EGF receptor remain unclear, though a critical role for receptor ubiquitination in this process has been proposed.

Equally important are various negative feedback loops originating from the activated terminal effector Erk that limit the maximal extent and duration of the signal. Prominent examples of these are the ligand-induced activation of the signal antagonist sprouty [10,11] and the inactivating phosphorylation of SOS by the activated Erk pathway [12]. By these mechanisms cells counteract cellular hyperactivation that could lead to an aberrant mitogenic response or malignant transformation. It also establishes an additional layer of regulation: Certain negative effectors may affect only specific downstream cascades, in this way determining the signaling outcome.

3. Mathematical models of basic EGF signaling

The first detailed large-scale mathematical model of the basic EGF pathway (and actually of intracellular signaling in general) was generated by Schoeberl et al. [13]. Their final system contains about 100 compounds participating in 150 reactions, described by a set of ordinary differential equations, which can be used for analysis in standard mathematical packages (e.g., MatLab [14]) or by dedicated biological simulation software (e.g., Gepasi [15]). A fully stochastic simulation based on this model has recently been published [16].

While this model did not aim for completeness, it provides a reasonably comprehensive, integrated picture of the system (Fig. 1). It contains a simple two-compartment implementation of receptor internalization and models both Shc-dependent and Shc-independent signal transduction. It has been widely referred to and forms the basis of several other computational analyses (e.g. [17–20]). Because this model is readily available on the internet (http://web.mit.edu/dllaz/egf_pap/) and is rightly considered a benchmark for other approaches, it will serve as our reference point for the following discussion.

Many other groups have developed computational models of EGF signaling, for example focusing on early signaling events, or using minimalistic descriptions of the pathway to identify general signaling properties. Some of these models approach the Schoeberl model in complexity (reviewed in [2]). Exciting recent developments towards integrated modeling are discussed below in the context of advances in our biological understanding of the EGF pathway.

4. Learning from mispredictions in computational models of EGF signaling

Computational models have been very useful in guiding experimental research into EGF biology. But to direct further development of more refined models it is useful to identify areas where current models fail. This should already indicate issues that will need special attention in future work.

The following short collection of exemplary cases focuses on the comprehensive signaling model by Schoeberl et al. [13]. Many of the same mispredictions would occur with some of the alternative models (if they do attempt to model the relevant phenomena at all). Three types of prediction errors seem to be of special interest.

4.1. Oversimplified molecular description

Sometimes, mispredictions in computational models arise from reasonable simplifications that were initially introduced to reduce the complexity of the description. This can lead to unexpected behavior of the model outside the conditions originally tested. At the same time, errors like this are probably the easiest to fix, once they have been identified. A striking example is provided by the Schoeberl model: It predicts that very soon after the initiation of signaling, the majority of Ras protein (98%) accumulates in a GTP-bound yet inactive state (Ras-GTP*), which is supposed to be unable to activate its downstream target Raf. This accumulation is essential for the transient response observed in the model.

However, such an inactive Ras-GTP* molecule (metabolite numbers 43 and 71 in the Schoeberl model) is not generally known to exist. Sydor et al. [21] explicitly argue against the existence of an inactive form of Ras-GTP and state that active Ras-GTP can activate multiple Raf molecules. Also, according to Muroya et al. [22] the level of GTP-bound Ras does not exceed 5–20%, and reaches these levels only transiently. As Ras-GTP is the central regulator of the pathway – and a potential target of regulatory feedback mechanisms – such a discrepancy will have important implications for the interpretation of the model.

4.2. Insufficient consideration of trafficking and spatiality

In many models that consider receptor trafficking explicitly, the only consequence of receptor internalization is its eventual degradation and consequent signal termination (e.g. [13]). Signal transmission, however, is supposed to happen at the same rate, independent of where the receptor is localized. This leads to the prediction that disruption of coated-pit endocytosis amplifies the Erk signal. This is in agreement with early studies showing that signal termination is achieved by ligand-induced receptor internalization.

However, more recently Vieira et al. [23] showed that MAP kinases are suppressed, not overactive, in conditionally endocytosis-defective cells (dynamin K44A mutants). Ringerike et al. [24] confirmed this finding and report that the mechanistic basis is a disruption of high-affinity EGF ligand binding in the mutant, even in the absence of endocytosis. Dynamin-deficiency and the consequent disruption of coated-pit endocytosis thus lead to a strong reduction of EGF-induced receptor autophosphorylation and downstream signaling, in contrast to the prediction of the model. The exact mechanism of these effects is still not sufficiently understood, and suitable in silico models will be helpful to differentiate between alternative hypotheses. As discussed below, the necessary inclusion of spatial details will be an important challenge for the next generation of computational models.

4.3. Incomplete biological knowledge

Both computational models and laboratory experiments agree that at saturating EGF concentrations EGFR overexpression leads to almost the same maximal Erk-PP signal, but with longer duration. However, Habib et al. [25] report that in the presence of low (i.e., physiological) EGF concentrations the signal is attenuated by EGFR overexpression in several cell lines, and they provide experimental evidence for a potential mecha-

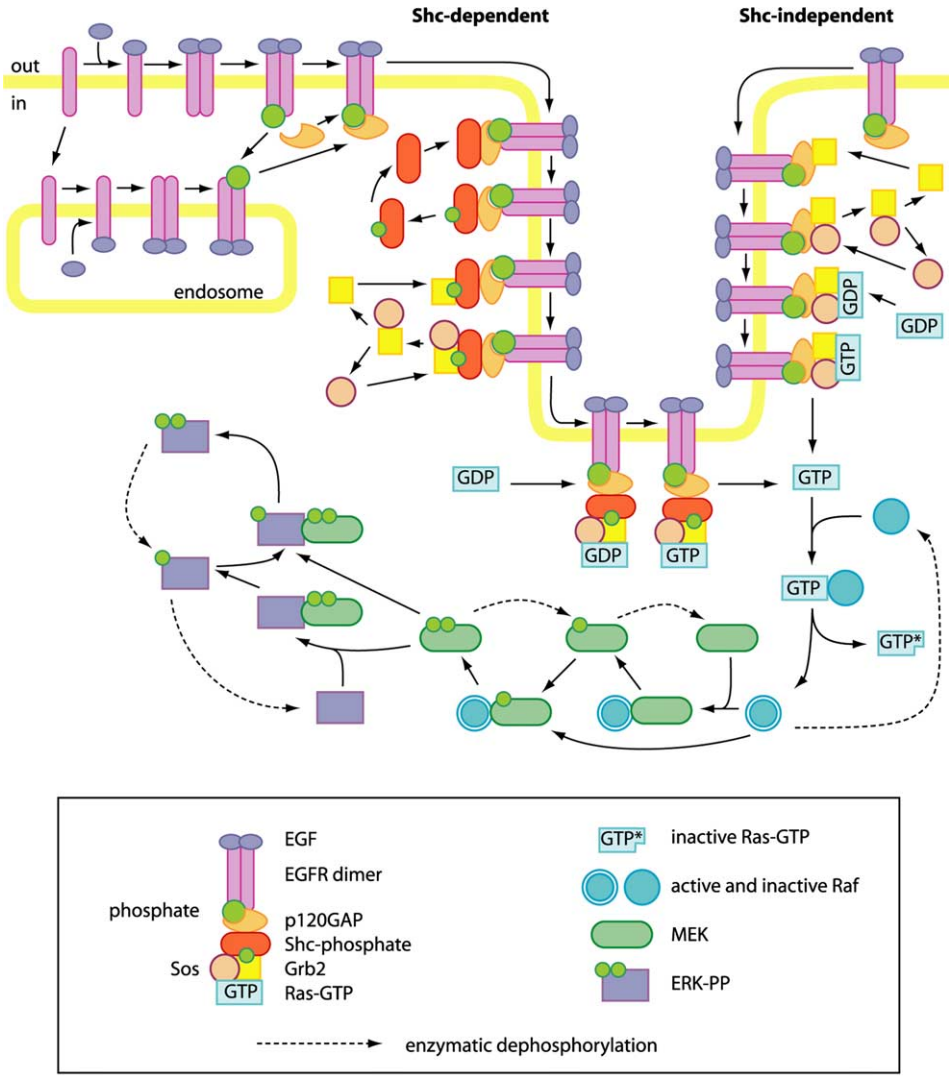

Fig. 1. Simplified diagram of the EGF signaling pathway encoded in the mathematical model by Schoeberl et al. [13]. The degradation reactions in the endosome, the reactivation of inactive Ras-GTP, and the clathrin mediated endocytosis are not shown in detail. Also, all species of active receptor complexes exist in a plasma-membrane and an endosomal version in the model. The enzymatic dephosphorylation reactions are shown in abbreviated form for clarity. Almost all reactions are modeled as reversible, but are shown as unidirectional here to emphasize the flow of information. For reaction numbers, exact equations and kinetic parameters, see the supplementary material of the original publication [13].

nism underlying this rather paradoxical effect. This attenuation is in direct contradiction model computations, which predicts that EGFR overexpression leads to amplified Erk-PP signal at low EGF concentrations. As Habib et al. discuss [25], the reason for this discrepancy may be the sequestration of Erk at the cell membrane in EGFR-containing complexes. Thus, this example may be a case of a "gap" in the description of the biological system. A number of recent advances in EGF research have highlighted additional important aspects of the EGF system that are commonly neglected in "classical" models. The next section will present the most important issues and describe emerging attempts to address these.

5. Current gaps in computational models of EGF signaling

5.1. Only one endosomal compartment is considered

Localization is the major emerging topic in the control and differential regulation of (not only) EGF receptor signaling (reviewed in [26]). In particular, exploiting the compartmentalization and functional specialization of the endocytic pathway can lead to a high order of regulation [27]. In some cases specific endosomal subcompartments, which are characterized by the presence of functionally active marker molecules, have been shown to provide specialized platforms for signal propagation [28,29]. In addition, recent findings point to a so far unappreciated role of distinct internalization routes for activated EGF receptor [9]. Is has been shown that at low EGF concentrations the receptor is directed to clathrin-coated pits, however, at concentrations higher than 25 ng/ml the receptor starts internalizing via the caveolar pathway in a switch-like manner. The molecular mechanism triggering this switch from the clathrin- to the caveola-mediated entry are not understood yet. However, it has been proposed that receptor ubiquitylation plays a crucial role since ubiquitylated receptor was predominantly found in caveolae. It was therefore suggested that the clathrin-mediated internalization pathway serves as "signaling route" because the receptor will be recycled back

to the cell surface, while at high EGF concentrations the caveolar pathway leads to permanent signal termination by lysosomal degradation of the ubiquitylated receptor. Thus, locality-dependent properties can contribute decisively to signaling kinetics, specificity, and outcome [30].

Haugh and Lauffenburger [31] in a seminal paper combined a simple model of (relatively) slow receptor trafficking and membrane dynamics with a quasi-steady-state reaction-diffusion model of the rapid interaction between the activated receptor and its immediate target. Both layers of the model are described by sets of differential equations with boundary conditions imposed by the experimental scenario.

Resat et al. [32] extended this approach by developing a more detailed stochastic description of trafficking and endosomal sorting in EGF signaling. Their work illustrates nicely the computational problems that arise when one moves from simple ordinary differential equations describing a homogenous aqueous solution to a realistic cell that is richly subdivided: Doing so immediately leads to an explosion of computational complexity of the model. Altogether the authors have to model about 275 compartments (various vesicles) and 13 000 reactions, despite considering only 23 distinct molecular species. They use a custom-built variant of the Gillespie Dynamic Monte Carlo algorithm [33] to perform realistic stochastic simulations in reasonable time. New mathematical techniques such as these, e.g. [16,33,34], are essential for the move towards increased realism in cellular models.

5.2. The complex protein interaction network is highly simplified

Highly diverse protein interaction networks and complexes occur at various levels in signaling pathways [35]. They can have regulatory functions both at the receptor level [36] and along the intracellular kinase cascade [37]. Much of the interaction, regulation and restriction of signaling pathways will happen via these scaffolds. Adaptor proteins which orchestrate the dynamic assembly of signalosomes at various places along the pathway are increasingly recognized as crucial regulatory sites which are important for basic systems behavior as well as human disease [38].

For example, at the proteome level, tight recruitment of proteins involved in ubiquitin-mediated receptor trafficking (Eps15, Hrs, c-Cbl, STAM1/2) is the single most strongly activated process in EGF-exposed HeLa cells [39]; quantitatively these protein show larger changes in activity than the majority of the classical kinase cascade members. Excluding them from an initial model may seem well justified by the lack of biophysical parameter data and the combinatorial explosion of the number of possible protein complexes [40]. However, this reduces the predictive power of such a model and may result in missing a major paradigm shift in cell biology.

5.3. Spatial aspects are neglected

Advances of imaging technology have lead to an accumulation of high-resolution spatio-temporal data on EGF signaling [41]. Some of these techniques make it possible to track the activation state of specific signaling molecules in living cells [42,43], sometimes at the level of single activated molecules [44].

Restricted diffusion of Ras is one of the earliest effects of EGF signaling [43], while at the same time a signaling wave spreads across the cell surface [45,46]. Understanding the interplay of restriction and diffusion of the signal is essential for interpreting the effect of EGF on cell shape and locomotion via local cytoskeletal remodeling [47], as well as on directional migration [48].

Localized autocrine ligand shedding provides an important mechanism for the sensing of mechanical deformations of the cell and their transduction to morphology-altering growth factor signaling [49]. Spatiality is also crucial for explaining the switch-like bistable response to threshold concentrations of ligand [50]. Another example of spatial distribution effects is the Ras/SOS/GAP module, which requires efficient recruitment to the membrane to achieve the observed catalytic activities [51]. Ichinose et al. [52] show how lateral clustering of ligand-bound and free EGF receptor leads to the spread and amplification of signal independent of cytosolic factors.

While compartmentalization can – with some difficulties – be integrated in the standard modeling approach, spatial aspects pose particular problems, as they require a transition from ordinary differential equations to partial differential equations with the spatial coordinates as additional variables (see, e.g. [31]). This not only necessitates additional information about compartment shapes and diffusion parameters, but more importantly specialized tools for the visualization and exploration of the models. Also, the need for quantitative kinetic data on processes like intracellular diffusion or the lateral phase separation of membrane components is a major problem.

Maly et al. [53] were among the first to address this issue by creating an EGF model that includes diffusion in three dimensions. They use this to analyze kinetic requirements of self-organization, i.e., local activity of EGF signal maintained by autocrine EGF release. Shvartsman et al. [54], in contrast, address spatiality at the level of entire cell assemblies. To this end they use an extremely 'lumped' minimal model, i.e. they combine very diverse blocks of signaling processes into single functional units that are modeled in toto, and use these to build a spatial model of EGF signaling, which includes intercellular communication and ligand diffusion, as well as (implicitly) transcription and translation. This model is presently specialized for Drosophila oocytes at a specific developmental stage. Further refinements would have to take changes in cell geometry and interaction into account.

5.4. Molecular diversity, feedback loops and crosstalk are oversimplified

Of course a large number of recent molecular advances are missing (in different combinations) from all available mathematical models. For instance, the model by Schoeberl et al. [13] does not include the recent finding, based on X-ray crystallography, that Ras-GTP can activate its nucleotide exchange factor SOS in a positive feedback loop [55]. This interaction might form an important additional mechanism underlying the switch-like behavior of EGF signaling.

Further examples include the regulatory role of molecules like Sprouty, which are part of an homoeostatic dual feedback loop in EGF signaling [11,56,57]; the critical function of Cbl and other ubiquitination-related factors in signal termination [58,59]; or the importance of molecular scaffolds like Ksr, which modulates the proliferation and oncogenic potential of cells by assembling molecules of the kinase cascade [60]. Discriminating between various regulatory mechanisms and determining their quantitative contribution requires sufficiently elaborate computational descriptions.

In addition, crosstalk with other signaling pathways can have important consequences for the biological outcome of EGF stimulation, e.g., interaction between an EGF/MAP kinase gradient and notch-like LIN-12 signaling is responsible for the precise positioning of cell fate decisions in nematode vulval morphogenesis [61], and transactivation of EGF signaling by G-protein coupled receptors is increasingly recognized as a common phenomenon underlying a wide variety of disease states (reviewed in [62]).

This additional complexity calls attention to another challenge for computational model building: While including the new details does not necessarily require a shift in modeling technology, it depends heavily on the availability of quantitative biophysical parameter data. Collecting the relevant information through laboratory experimentation is currently the major bottleneck of systems biology. Each additional component that can be incorporated in the model immediately adds a number of potential simple *interventions* (gene knock-down, overexpression, inhibition), thus critically increasing the experimental flexibility in testing the computational predictions.

6. Conclusions and perspectives

Mathematical models of EGF signaling make a valuable contribution to computational cell biology. Nonetheless they contain gaps, in particular with respect to cellular mechanisms that have recently moved to the front of EGF research. To address this issue, the next generation of computational models will have to incorporate the exciting new findings on the molecular mechanisms of signal perception, transmission, and termination. In many cases, this will require a move towards a more integrated, spatially rich description of the system and the adoption of other modelling techniques beyond ordinary differential equations. Several recent papers have already shown the feasibility of such approaches [32,53,54] and software platforms that can deal with the new complexity of models are now becoming available (SmartCell, [63]; Virtual Cell, [64]). Only by including sufficiently realistic detail will the generated models attain general relevance and enough predictive power to suggest new experimental or therapeutic interventions.

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